Effect of Seizures Induced by Intra-Amygdaloid Kainic Acid on Kainic Acid Binding Sites in Rat Hippocampus and Amygdala

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Abstract: [^{3}H]Kainic acid binding sites with a slow dissociation rate in the rat limbic system were investigated in detail. Extensively washed membranes prepared from the hippocampal formation and from the region comprising the amygdala and the piriform cortex yielded nonlinear Scatchard plots. Microdissection showed that the high-affinity component (affinity constant around 1 nM) was present in the hippocampal CA3 region (4.2 fmol/mg wet tissue) and the amygdaloid complex (4.6 fmol/mg wet tissue), whereas the remaining part of the hippocampal formation and the piriform lobe contained the low-affinity component (affinity constant 5–20 nM; 11.6 and 11.3 fmol/mg wet tissue, respectively). In the lateral + medial septum we detected only the low-affinity component. Severe limbic seizures, induced by unilateral injection of 0.7 or 0.8 μg kainic acid in 0.3 μl of phosphate-buffered saline into the amygdala, reduced kainic acid binding sites in the ipsilateral amygdala and CA3 region. The decline of kainic acid binding sites in the injected amygdala was followed by a similar effect in the contralateral amygdala ("mirror focus") and later by a moderate loss also in the contralateral CA3 region. Kainic acid receptor autoradiography demonstrated that binding sites were lost from the stratum lucidum in hippocampus. Septal lesion had no effect on kainic acid binding sites in the hippocampus. Comparison with previous results on the histopathological changes after this lesion shows that high-affinity kainic acid binding sites are preferentially located on neurons that undergo selective degenerations after severe kainic acid-induced seizures. **Key Words:** Kainic acid—Binding sites—Limbic seizures—Hippocampus—Amygdala. **Berger M. L. et al.** Effect of seizures induced by intra-amygdaloid kainic acid on kainic acid binding sites in rat hippocampus and amygdala. *J. Neurochem.* 47, 720–727 (1986).

Kainic acid (KA), a rigid structural analogue of the putative excitatory neurotransmitter glutamic acid, provokes on systemic and central injection limbic seizures with brain damage in which the hippocampus, amygdala, and other limbic structures play a central role (Ben-Ari et al., 1980; 1981; Lothman and Collins, 1981; Sperk et al., 1983). This experimental model is at present widely accepted as a good animal model for human temporal lobe epilepsy (Nadler, 1981; Ben-Ari, 1985). The mechanism of interaction of KA with neuronal tissue is under intense investigation, since its clarification could provide some insight toward a better understanding of the etiology of human temporal lobe epilepsy. Recent results from our laboratory were compatible with the hypothesis that high-affinity KA binding sites in limbic structures are involved in the induction of seizures by the neurotoxin (Ben-Ari et al., 1984; Berger et al., 1984). We had differentiated these high-affinity sites from low-affinity ones by selective experimental conditions that allow the observation of high-affinity binding sites only. However, we still observed nonlinear Scatchard plots (Berger et al., 1984), indicating that in some brain regions high-affinity KA binding consisted of two components with different affinities. The present study was undertaken to shed more light on this apparent binding site heter-

Abbreviations used: B_{max}, maximal number of binding sites; DG, dentate gyrus; K_D, dissociation constant; KA, kainic acid.

ogeneity and to follow up the time course of KA receptor changes in several brain regions after severe limbic seizures, induced by intra-amygdaloid injection of KA.

MATERIALS AND METHODS

The hippocampi of nine male Wistar rats were dissected out from unfrozen brains and subdissected on an ice-cold glass dish into the "CA3 part" and the remaining "DG/CA1 part," containing the dentate gyrus (DG), the CA1 region, and a part of the subiculum (Fig. 1A). We chose this particular placement of the longitudinal knife cut after preliminary experiments with slices taken from frozen hippocampi. The thawed slices were stained with a droplet of methylene blue solution and observed through a low-power microscope. In that way we verified that a cut along the minor fissure close to the fimbria yielded the desired separation between CA3 part and the rest. Tissue blocks containing the amygdaloid complex and the overlying part of the piriform lobe were obtained by three knife cuts along well-defined neuroanatomical landmarks (Figs. 1B and 2), also from unfrozen brains. The amygdaloid complex was isolated from the piriform cortex from the ventral side along a border line that could clearly be seen. After removal of the piriform lobe, the latero-dorsal cutting edge coincided with the myelin layer under the cortex. Septal tissues were pooled from 24 rats and used for two Scatchard analyses. Tissues were stored at −20°C for up to 9 days.

For lesion studies, five rats received 0.8 μg KA (dissolved in 0.3 μl phosphate-buffered saline) into the right amygdala within 5 min under equithesin anesthesia; the needle was taken out after 5 min. After recovery from anesthesia, the animals developed severe limbic motor seizures and received 20 mg/kg diazepam 2–3 h after sei-

zure onset to prevent death. If necessary they received a second dose a few hours later. In six rats we verified that these doses of diazepam injected intraperitoneally had no effect per se on KA binding sites.

In five rats, the septum as a whole was lesioned unilaterally by electrocoagulation; this resulted in the almost complete loss of acetylcholinesterase from the hippocampus. KA binding sites were analyzed only in the hippocampus ipsilateral to the lesion.

Rats were killed 15 days after the intra-amygdaloid injection of KA and 12 days after septal lesion by stunning and decapitation. Hippocampal subregions, amygdalae, and piriform cortices were dissected as described above and stored at −20°C. Corresponding regions from two times three of the nine control animals were dissected and processed together with the lesioned tissues. For membrane preparation, tissues were thawed and homogenized with a motor-driven glass-Teflon Potter in 50 parts 50 mM Tris-acetate buffer (pH 7.0). After two centrifugations (10 min, 20,000 g) and resuspensions in fresh buffer (Ultra Turrax), membranes were incubated for 15 min at 37°C to liberate endogenous compounds that could interfere. After cooling, they were centrifuged once more, resuspended in fresh buffer, and stored at −20°C for 1–7 days.

In an additional series of experiments, we studied KA binding sites 2 and 5 days after intra-amygdaloid injection of the toxin. In these experiments, 0.8 μg KA killed three out of four rats; thus, we were forced to reduce the dose to 0.7 μg. By that, nine out of 10 animals survived. Rats were killed 2 days (n = 5) and 5 days (n = 4) after KA injection and limbic subregions dissected as described above, but processed immediately for membrane preparation, without freezing. Final suspensions were frozen in a Dewar flask with dry ice, after 18 h transferred to −80°C, and analyzed after 1–40 days. Corresponding regions from two times three untreated control rats were
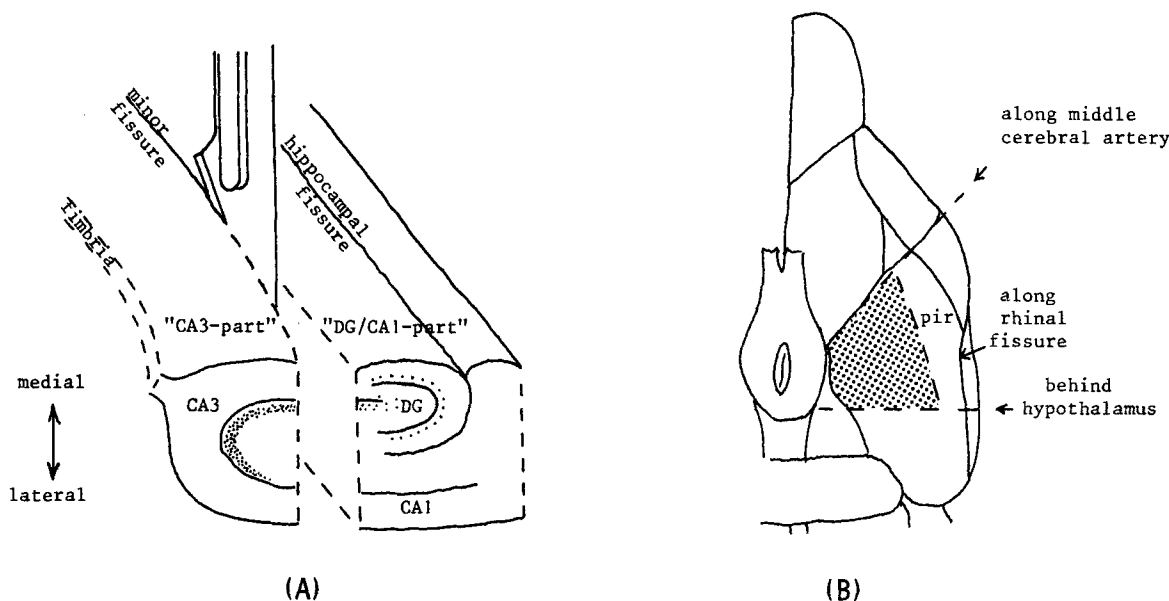

(A)   (B)

FIG. 1. Microdissection of the hippocampal formation (**A,** schematic coronal section) and of the amygdala + piriform lobe (**B,** schematic dorsal view). The hippocampus was excised from unfrozen brains and separated with a scalpel into two strips along the fissure close to the fimbria. The smaller part (approximately 15 mg) contained the main part of the CA3 region. The amygdala was isolated in situ from surrounding tissue by three vertical knife cuts indicated by dashed lines in (B); a fourth incision was conducted horizontally along the rhinal fissure. The boundary between the amygdala and the piriform cortex could be seen, since the amygdala had a darker appearance (dotted area). One amygdala weighed 10–15 mg.
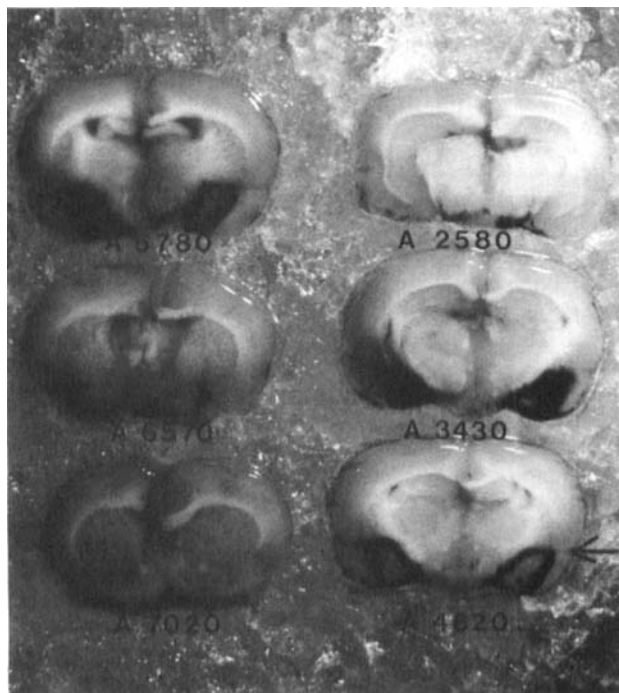
FIG. 2. Coronal slices (approximately 1 mm) cut from a rat brain after dissection of the amygdala. Both amygdalae were dissected as illustrated in Fig. 1, dipped into a methylene blue solution, and reinserted into the brain. Note the clear separation from the caudal part of the striatum (arrow). Anterior coordinates according to König and Klippel (1963).

dissected the same day and carried through all preparation and storage steps together with the lesioned material. Thus, both the materials from septal lesions and from each survival time after intra-amygdaloid KA injection could be compared with identically processed control material. We had some indications that the duration of the membrane preparation and the exact freezing and storage conditions had some effect on the binding parameters. Table 1 illustrates that the binding parameters varied from one preparation to another (especially with DG/CA1 membranes).

For binding assay, the stored suspensions were thawed and centrifuged once more; thus, all membranes were washed twice after the 37°C preincubation, once more than in previous studies (Berger et al., 1984). This additional washing step resulted in slightly lower affinity constants, suggesting a more complete removal of endogenous interfering compounds. HPLC amino acids analysis by the method of Schmid et al. (1980) after the 37°C incubation and centrifugation indicated free glutamic acid and γ-aminobutyric acid (GABA) concentrations in the supernatant around 1 μM (striatal membranes; Berger and Reither, 1982, unpublished observation). Scatchard analyses of control membranes and membranes after septal lesion were performed in Beckmann Biovals (triplicates), using 10 different ligand concentrations ranging between 0.25 and 128 nM [³H]KA (5 or 2 Ci/mmol, Amersham) for total binding and four concentrations for nonspecific binding (in the presence of 10 μM unlabelled KA); the remaining values for nonspecific binding were obtained by linear interpolation of the saturation isotherms. For analyses of material after intra-amygdaloid KA injection,

only five ligand concentrations were chosen for total binding, and three concentrations for nonspecific binding (again in triplicate). Only with this simplification was it possible to analyze ipsi- and contralateral lesion material together in one assay procedure; control material was assayed immediately thereafter or the next day. Incubations were conducted for 90 min on ice and stopped by the addition of a 5-μl droplet of 2 mM unlabelled KA to an incubation volume of 1 ml (resulting in a final concentration of 10 μM in all vials, since this drop was not added to nonspecific binding samples). All samples were centrifuged 1.5 min later; this was the minimum period required for the reproducible manipulation of 52 samples and by far enough time to allow for the complete displacement of [³H]KA from fast dissociation rate sites (Berger, manuscript in preparation). Pellets were rinsed superficially by 2 × 4.6 ml diluted ice-cold buffer, solubilized in 300 μl Protosol (New England Nuclear; 2 h, 55°C), and the radioactivity counted after addition of 50 μl concentrated acetic acid and 3 ml Aqua-sol-2 (New England Nuclear) or Rotiscint-22 (Roth, Karlsruhe, F.R.G.) by liquid scintillation counting.

Ten-point Scatchard plots were analyzed by linear regression as well as by Gauss-Newton nonlinear regression, performed on an HP 41 CV computer, with a program adapted from the computer center of the University of Vienna (Robinson, 1977). For five-point Scatchard plots, we used linear regression only, exploiting the possibility of an estimate for standard deviations (Sachs, 1969); the values so derived for the binding parameters were statistically treated as means of triplicates (five points minus two parameters) and used for unpaired Student's t test. By that, only results obtained from simultaneously dissected, prepared, and stored material were compared to each other.

For KA binding site autoradiography, three rats with amygdaloid KA lesion and two rats with septal lesion were used. After 15 and 12 days, respectively, brain slices were prepared as described elsewhere (Berger and Ben-Ari, 1983; Berger et al., 1984). Incubations were conducted in the presence of 20 nM [³H]KA (5 Ci/mmol) and stopped as described above for membrane suspensions by the addition of unlabelled KA and subsequent washing. Nonspecific binding was omitted, since earlier studies revealed no detectable difference between nonspecific binding and film background at that ligand concentration (Berger and Ben-Ari, 1983). Dried slide-mounted slices were exposed to ³H-Ultrofilm for 3 months.

RESULTS

KA binding sites in microdissected control tissue

In membrane preparations containing the main part of the CA3 region (Fig. 1A), we found both high- and low-affinity components of KA binding with a slow dissociation rate, whereas in the remaining part of the hippocampal formation (DG/CA1 part) we detected only the low-affinity component (Fig. 3A and B). Isolation of the amygdaloid complex from the piriform lobe yielded similar results: both components were detected on amygdaloid membranes, but only low-affinity sites (sometimes with a small contamination from high-affinity
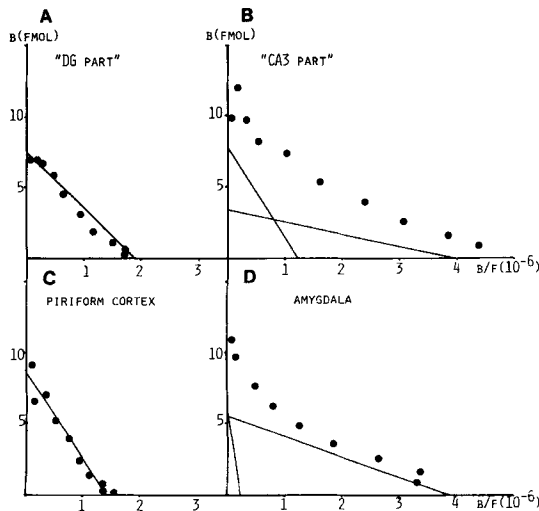
FIG. 3. Scatchard analyses of slow dissociation rate KA binding to membranes prepared from limbic subregions. Binding parameters as obtained by computer analysis are indicated in Table 1 (superscript a). B, specifically bound [³H]KA (fmol/mg wet tissue); B/F, ligand bound/free ligand concentration (unit: 1×10^{-6}).

ones) in the piriform lobe (Fig. 3C and D). From septal membranes, two experiments yielded linear Scatchard plots, with affinity constants around 10 nM (Table 1) corresponding to the low-affinity component only. This was in agreement with earlier observations (Berger et al., 1984). The detailed results are summarized in Table 1 in chronological order of

TABLE 1. *Parameters of slow dissociation rate KA binding in several subregions of the rat limbic system (nonlinear regression)*

Region	K_{D1}	$B_{max\ 1}$	K_{D2}	$B_{max\ 2}$	n
Septum	—	—	10.8	5.53	12
CA3 part	1.30	4.30	8.60	7.70	3
DG/CA1 part	—	—	5.97	10.7	3
CA3 part	2.40	7.10	64.0	4.00	3
DG/CA1 part	—	—	4.33	8.88	3
Septum	—	—	10.0	5.20	12
CA3 part[a]	0.88	3.47	6.40	7.98	3
DG/CA1 part[a]	—	—	4.01	7.51	3
Amygdala[a]	1.43	5.59	24.2	6.29	3
Piriform cortex[a]	—	—	6.08	8.72	3
CA3 part[b]	0.87	2.61	7.55	6.58	3
DG/CA1 part	—	—	13.2	16.1	3
Amygdala	1.27	3.66	30.4	10.8	3
Piriform cortex	0.59	1.80	7.64	12.5	3
CA3 part	1.24	3.59	11.6	5.31	3
DG/CA1 part	—	—	14.9	14.7	3
Piriform cortex	0.24	0.49	8.53	12.6	3

K_{D1}, $B_{max\ 1}$, affinity constant (nM), and maximal number of binding sites (fmol/mg wet tissue) of the high-affinity component; K_{D2}, $B_{max\ 2}$, respective parameters of the low-affinity component; n, number of rats. Horizontal lines separate results obtained from different groups of animals.

[a,b] Illustrated in Figs. 3 and 6, respectively.

the experiments. Regions that were dissected from the same brains are grouped together (we did not analyze all limbic regions in every experiment). Some of these results are illustrated as Scatchard plots in Figs. 3 and 6 (indicated in the table by superscripts a and b, respectively).

Fate of KA binding sites after selective lesions

Injection of 0.7 or 0.8 μg KA into the right amygdala produced severe limbic motor seizures. The animals were continuously masticating and foaming from the mouth; we observed right forelimb clonus, barrel rotation, rearing, and loss of postural control (Ben-Ari et al., 1980; Tremblay et al., 1983). Injection of 20 mg/kg i.p. diazepam immediately interrupted their seizures; sometimes a second dose was needed to prevent reappearance of the seizures. At 15 days after the KA-induced seizures, we found strong reductions of specific KA binding sites with slow dissociation rate at the site of injection, with no significant change in the affinity constant (K_D; Fig. 4). The maximal number of binding sites (B_{max}) was also reduced in the contralateral amygdala (open columns in Fig. 4). The most pronounced loss of slow dissociation rate KA binding sites occurred in the CA3 part of the ipsilateral hippocampal formation. KA binding in the contralateral CA3 subregion was also slightly affected (p < 0.05); in both hemispheres, K_D values in CA3 were normal. Twelve days after complete destruction of one septum (accompanied by a large loss of acetylcholinesterase in the hippocampus), membranes prepared from the CA3 part still yielded nonlinear Scatchard plots very similar to simultaneously prepared control membranes (not illustrated). Thus, the removal of one important hippocampal afferent did not cause the disappearance of one of the KA binding components. We completed our lesion studies by KA binding site autoradiography. After septal lesion (again 12 days), no qualitative changes in the hippocampal distribution of KA binding sites were detected (not illustrated). At 15 days after intra-amygdaloid KA injection, KA binding sites in the stratum lucidum of the ipsilateral hippocampus were still detectable in the temporal part (Fig. 5C), but almost completely lost from the septal part (Fig. 5A), in keeping with the septo-temporal gradient of neuronal vulnerability to intra-amygdaloid injection of KA (Ben-Ari et al., 1980). Binding sites in the stratum moleculare of the DG seemed to be less affected, in agreement with the study of Monaghan and Cotman (1982).

Time course of KA receptor changes after amygdaloid KA lesion

Binding studies 2 days and 5 days after injection of 0.7 μg KA into the right amygdaloid complex revealed a much faster reduction in KA binding sites than seen, e.g., in the striatum after local injection of the neurotoxin (Coyle et al., 1978; Beaumont et
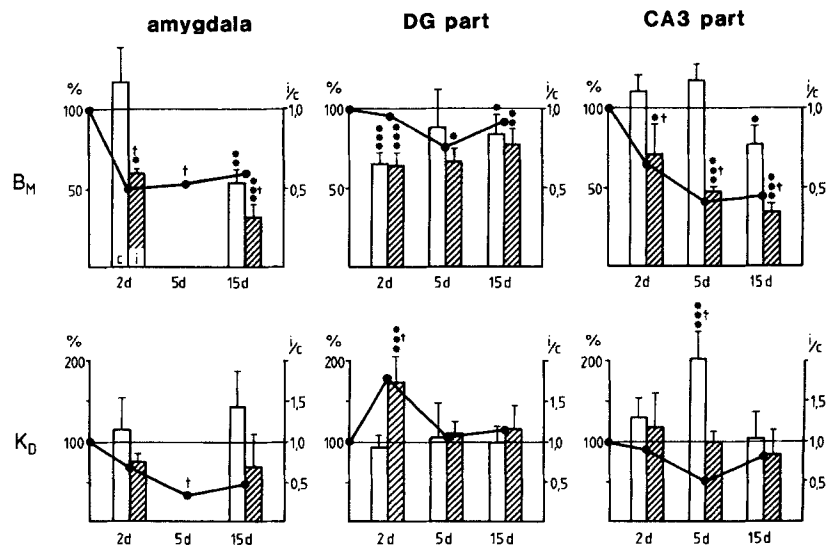
FIG. 4. Time course of changes in KA binding sites with slow rate of dissociation after injection of KA into the right amygdala. Maximal numbers of binding sites (B_{max}, above) and affinity constants (K_D, below), as obtained from linear regression on five-point Scatchard plots, are expressed as percentage of respective values from coprocessed control tissue. Hatched columns, ipsilateral hemisphere (i); open columns, contralateral hemisphere (c); filled circles, ratio ipsi- to contralateral. *,**,***Significantly different from control with p < 0.05, p < 0.01, p < 0.001; †significantly different from other hemisphere with p < 0.05.

al., 1979). Already 2 days after the KA-induced seizures, significant losses in KA binding sites (B_{max}) occurred ipsilaterally to the injection site in all regions analyzed (amygdala, DG/CA1 part, and CA3 part; Fig. 4). In the DG/CA1 part the reduction in B_{max} 2 days after the lesion was bilateral, and ipsilaterally accompanied by a loss in affinity (i.e., elevation of K_D). With longer survival times, these changes did not proceed, and K_D values returned to control levels. In contrast, the loss of KA binding sites in the ipsilateral CA3 mossy fiber terminal zone continued even between 5 and 15 days postlesion (Fig. 4). Binding sites in the contralateral amygdala showed a similar decrease up to day 15, accompanied by a transitory increase in K_D at day 5 (14.5 nM against a normal value of 3-4 nM; p < 0.05, if compared to the ipsilateral amygdala, where the K_D was 4.65 nM; Fig. 4). Unfortunately, amygdaloid control membranes in the 5-day experiment showed for some unknown reason no binding capacity at all (note the blank space in Fig. 4 at day 5). Therefore, a reliable reference to amygdaloid control material was not possible in this 5-day experiment, and we indicated only the ratio ipsi- to contralateral (i/c, Fig. 4). The development of an amygdaloid "mirror focus" 5-15 days after the KA injection was correlated with a late reduction in KA binding sites in the contralateral CA3 part. After 2 days and 5 days, there was still no reduction in CA3 B_{max} values. A transient loss in affinity is reflected by a doubling of the K_D value at day 5 (Figs. 4 and 6).

DISCUSSION

Recently, we studied the ontogeny of the seizure-brain damage syndrome induced by systemic injection of KA into rats and established in parallel the postnatal development of KA membrane binding

sites in the rat limbic system (Ben-Ari et al., 1984; Tremblay et al., 1984; Nitecka et al., 1984; Berger et al., 1984). In the binding assays, we differentiated between binding sites with slow and fast rates of dissociation, respectively (London and Coyle, 1979). However, individual Scatchard analyses of KA binding to these respective subpopulations of sites indicated still heterogeneity of the ligand–receptor interaction in the hippocampal formation and in the amygdala + piriform lobe in adult rats, whereas septal membranes seemed to contain only the component of lower affinity (Berger et al., 1984). The question arose as to whether the apparent binding site heterogeneity in the former two regions might be due to the heterogeneity of the tissue used for membrane preparation. Our results obtained in the present study on microdissected subregions of the respective structures indicate that this really was the case. Thus, the major part of the hippocampal formation, which did not contain the CA3 subfield, yielded Scatchard plots without the high-affinity component. The same was true for the piriform cortex (Fig. 3A and C). Only the CA3 part and the amygdala proper contained KA-binding sites with K_D values around 1 nM (Fig. 3B and D; Table 1). However, also in these regions we still found >50% of binding sites with lower affinity for the ligand. This could be due to a further heterogeneity of these subregions. To address this question, we studied the effects of selective lesions on K_D and B_{max} of KA binding with slow dissociation rate in limbic subregions. Electrocoagulation of the septum and KA injection into the right amygdala were chosen since they allow the observation of changes distant from the directly affected locus, avoiding the problem of local nonspecific reactions. Septal lesion had no effect on KA binding to hippocampal membranes. Intra-amygdaloid injection of KA, causing widespread
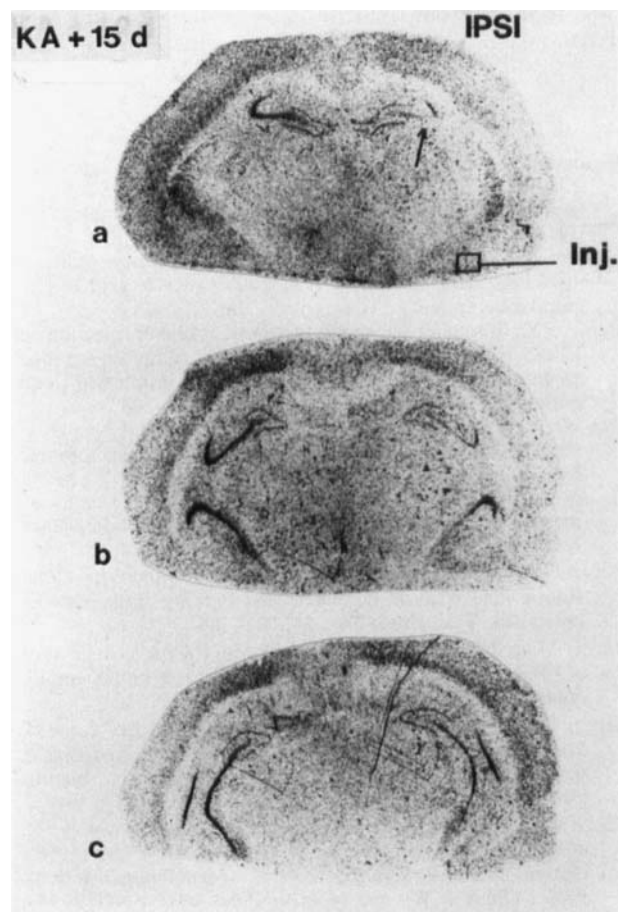
FIG. 5. Autoradiographs of 16-μm slices taken from rat brains 15 days after injection of 0.8 μg KA into the right amygdala (Inj.) and labelled in vitro with 20 nM [³H]KA (5 Ci/mmol); exposed 3 months to ³H-Ultrofilm. KA binding sites in the stratum lucidum of the ipsilateral hippocampus are strongly decreased, especially in proximity to the septal pole (arrow).
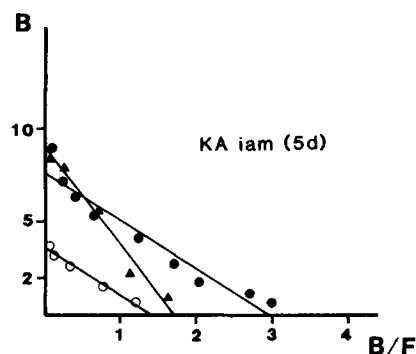

FIG. 6. Scatchard analyses of high-affinity KA binding to membranes prepared from CA3 part, 5 days after injection of 0.7 μg KA into the ipsilateral amygdala. (○), Ipsilateral side; (▲), contralateral side; (●), coprocessed control tissue. Binding parameters as obtained from linear regression, expressed in the form $K_D \pm$ SD, $B_{max} \pm$ SD (v) (K_D in nM, B_{max} in fmol/mg wet tissue; v, degrees of freedom) were as follows: ipsilateral 2.57 ± 0.33, 3.55 ± 0.22 (3); contralateral: 5.24 ± 0.88, 8.88 ± 0.83 (3); control: 2.58 ± 0.65, 7.61 ± 1.06 (7). Graph units as in Fig. 3.

neuronal degenerations in the whole limbic circuitry (see Ben-Ari et al., 1980), had permanent impact on the maximal number of KA binding sites with slow kinetics in most regions analyzed; however, in none of the affected regions did Scatchard analysis indicate the specific loss of one component of high-affinity binding only. Thus, our lesion studies do not provide any evidence for the hypothesis that high- and low-affinity components of KA binding sites with a slow rate of dissociation could be located on different elements. If they are, KA-induced seizures destroy both of them.

Interestingly, we did not find any brain region with only the high-affinity component (including the striatum, not shown), but in many cases only the low-affinity component was observed (septum, DG/CA1 part, piriform cortex). A gradual conversion of high- to low-affinity KA binding sites, departing from an originally homogeneous high-affinity population with K_D around 1 nM during the

membrane preparation procedure, should be taken into consideration as a possible source of apparent heterogeneity. Indeed, we have seen such a conversion in vitro during prolonged incubation of striatal and hippocampal membranes on ice ($t_{1/2}$ = 2–3 h, Berger, unpublished observation). Given this, it might well be the case that immediately after homogenization of such tissues as the CA3 part, the amygdala, or the striatum KA binding sites with a slow dissociation rate were almost completely in the "1 nM form."

The detailed time course of KA binding site reduction in various limbic structures after KA-induced seizures suggests that high-affinity KA binding sites in the limbic system are located on elements subjected to progressive seizure-induced degeneration. The first severely affected region was the DG/CA1 part, with the maximal reductions in the number of binding sites by 40% 2 days after the intra-amygdaloid KA injection, in both hemispheres. Since these reductions did not proceed after day 5, they may reflect adaptive changes to overactivity rather than neuronal degeneration. Small reductions in KA binding sites remaining 15 days after the seizures could reflect neuronal degeneration in the medial edge of the CA3 ("CA4"), which reaches into the hilus and is partly contained in this heterogeneous tissue. This part of the CA3 is notoriously susceptible to KA-induced seizures (Ben-Ari et al., 1980; Nitecka et al., 1984). The classic decline of KA binding sites in the ipsilateral CA3 region was progressive from 2 days to 15 days post-lesion and resulted in their virtual disappearance in proximity to the septal pole (Fig. 5). Since the well-established histopathological effect of intra-amygdaloid KA injections is a similar loss in CA3 pyramidal cells (Ben-Ari et al., 1980), it is

tempting to conclude that high-affinity KA binding sites are situated on these neurons. However, also after destruction of the mossy fiber afferents KA binding sites are lost from CA3 (Monaghan and Cotman, 1982; Tremblay, Repressa and Ben-Ari, unpublished observations). Taking into account the losses of mossy fiber endings reported after destruction of their target cells (Nadler et al., 1983), it must at present remain an open question whether KA binding sites in the stratum lucidum are located pre- or postsynaptically.

The causal relationship between the loss in KA sites in the CA3 and in the amygdala is underlined by the events in the contralateral hemisphere. Contralaterally to the intra-amygdaloid injection, the KA receptor level in CA3 remained normal up to day 5, when the amygdala just began to lose binding sites. By day 15, amygdaloid KA binding was strongly reduced also contralaterally to the principal lesion ("mirror focus," well known from histopathologic and metabolic studies; Ben-Ari et al., 1980; Tremblay et al., 1983; see also Schwob et al., 1980, who injected KA into the piriform cortex), and the number of binding sites in the corresponding CA3 region had fallen below the control value. Strikingly, both these late reductions in the maximal number of binding sites were preceded by a transient loss of affinity for the ligand, which might be indicative of the loosening of synaptic contacts during phagocytosis of degenerated neurons.

In summary, our data demonstrate that a certain component of KA binding sites with very high affinity for the neurotoxin is located in limbic subregions, which occupy crucial positions in the KA-induced seizure-brain damage syndrome. The maintained heterogeneity of slow dissociation rate KA binding in these regions could be due to post-homogenization changes rather than to in situ heterogeneity of sites. After severe limbic seizures, induced by intra-amygdaloid KA injection, the sequential decline in KA binding sites with a slow dissociation rate in limbic structures ipsi- and contralateral to the injection site agrees in its time course with the removal of degenerated neurons by phagocytosis. In particular the late changes seen in the contralateral hemisphere indicate the existence of endogenous toxic mechanisms, which are almost as destructive as the direct action of the neurotoxin, especially for elements bearing very high-affinity KA receptors. We suggest that these sites could be involved in the etiology and/or histopathology of certain forms of human epilepsy.

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